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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
ETSURO OGATA ET AL	: EXAMINER: HUYNH
SERIAL NO: 10/019,501	:
FILED: DECEMBER 31, 2001	: GROUP ART UNIT: 1644
FOR: AGENT FOR AMELIORATING LOW VASOPRESSIN LEVEL	:

DECLARATION UNDER 37 C.F.R. § 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

I, Etsuro Onuma, hereby declare as follows:

1. I am a Japanese citizen, receiving mail at Product Research Dept., Kamakura Research Labs., Chugai Pharmaceutical Co., Ltd., 200 Kajiwara, Kamakura, Kanagawa, 247-8530, Japan.

2. I am presently employed as scientist in the Chugai Pharmaceutical Co., Ltd. A copy of my Curriculum Vitae is attached.

3. I am a co-inventor of the subject matter of the above-identified U.S. Patent application. I am familiar with the specification and pending claims, and with the prosecution history of the application.

4. The following experiments were conducted by me or under my supervision and control. The experimental results demonstrate that anti-PTHrP antibody when administration resulted in an increase in vasopressin. However, if administered Alendronate (sodium [4-amino-1-hydroxy-1-(hydroxy-oxido-phosphoryl)- butyl]phosphonic acid trihydrate), this did not effect vasopressin

levels in the blood. Alendronate is a typical therapeutic agent for treating humoral hypercalcemia of malignancy (HHM) thus indicting that therapeutic effects on HHM bears no immediate relationship to vasopressin in the blood.

5. Using a hypercalcemia model animal (human tumor implanted nude rat model), a humanized monoclonal antibody against PTHrP and alendronate that is one of bisphosphonates were examined for their effects on blood vasopressin level.

6. <METHOD>

As a model animal, a nude rat implanted with human large cell lung carcinoma LC-6 [purchased from the Central Institute for Experimental Animals] was used. It is known that a nude rat implanted with human large cell lung carcinoma LC-6 shows an increased blood calcium level as increasing the tumor volume and develops weight loss and so on. In this example, this hypercalcemia model animal was used to examine blood vasopressin level by comparison with a normal rat, and to evaluate effects of the humanized monoclonal antibody or bisphosphonates (alendronate) on blood vasopressin level.

7. The hypercalcemia model animals were produced and divided into groups in the following manner. The human large cell lung carcinoma LC-6 were maintained in vivo in nude mice (BALB/c-nu/nu) (CLEA Japan, Inc.) , and then finely cut into 3-mm cube of blocks. The resulting tumor blocks were subcutaneously implanted into each of the rats at the lateral region at a ratio of one piece per rat. As rats, 4-weeks-old male F344/N Jcl-rnu nude rats (CLEA Japan, Inc.) were purchased and acclimatized for 10 days. The resulting 6-weeks-old rats were implanted with

the tumor. About a month and a half after the implantation (day 41), the rats with increased blood calcium levels and reduced body weights were used as hypercalcemia model animals for pharmacological test. Nude rats showing hypercalcemia were divided into three groups (n=13 rats per group, respectively)so that blood calcium levels and body weights of the rats in the individual groups were averaged. And the other group was normal rat (non-tumor bering nude rats)(n=7).

8. The humanized monoclonal antibody against PTHrP was administered to each of a group of the hypercalcemia model animals thus prepared via the caudal vein, once a week (6 times in total), at a dose level of 3.0 mg/kg. Or the bisphosphonates (alendronate) was administered to each of a group of the hypercalcemia model animals thus prepared via the caudal vein, twice a week (12 times in total), at a dose level of 2.5 mg/kg. As a control, phosphate buffered saline (PBS) was administered to each of another group of the hypercalcemia model animals via the caudal vein once a week.

9. On day 42 after the administration of the above antibody, the bisphosphonates (alendronate) or PBS, blood was collected from the descending aorta, followed by separation of plasma and serum in an EDTA-containing tube and a Separapid tube, respectively. The determination of blood vasopressin levels was performed by RIA method using plasma.

10. <<RESULTS>>

The results of above experiments are shown in the attached document. The hypercalcemia model animals were shown to have decreased blood vasopressin levels. That is, the concentration of vasopressin in blood decreased to 289.5±57.2 (pg/mL) in PBS-administrated group. Note that

concentration of vasopressin in blood was 1064.2±333.1 (pg/mL) in normal animal. The concentration of vasopressin in blood increased to711.1±141.3 (pg/mL) with administration of anti-PTHrP antibody in the hypercalcemia model animals. However, there was no improvement in blood vasopressin levels by administration of the bisphosphonates (alendronate). Note that concentration of vasopressin in blood was 241.9±62.2 (pg/mL) in the hypercalcemia model animals that the bisphosphonates (alendronate) had been administrated

11. <<DISCUSSION>>

Although the bisphosphonate (alendronate) is well-known as a therapeutic agent for hypercalcemia, the experiments clearly indicate that the bisphosphonate (alendronate) does not have a therapeutic effect on improvement in blood vasopressin levels. In contrast, the anti-PTHrP antibody had a therapeutic effect on improvement in blood vasopressin levels as well as therapeutic effect for hypercalcemia. Thus, in my view the treatment of hypercalcemia bears no immediate relationship to vasopressin levels in blood. That is, the present application is the first to show that PTHrP causes a decrease in vasopressin level in blood, and that administration of anti-PTHrP antibody increase in vasopressin levels in blood.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any

patent issued thereon.

Date: 08/13/2008 By Etsuro Onuma
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